DESIGNING THE NEXT GENERATION CD33-TARGETING ADC: IMGN779, SELECTED FOR POTENCY, NOVEL MECHANISM AND PRECLINICAL TOLERABILITY, WITH HIGH ACTIVITY IN DISSEMINATED AML MODELS AND MULTI-DOSE REGIMENS

P526

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IMMUNOGEN

BACKGROUND

Antibody-drug conjugates (ADCs) targeting CD33 are promising therapeutics in AML, where a challenge is achieving efficacy while maintaining tolerability. Here, we report the payload/ linker design and selection resulting in a high-Therapeutic Index (TI) CD33-targeting ADC with a favorable preclinical toxicity profile across multiple species and antitumor activity in disseminated AML models and multi-dose regimens. IMGN779, the final ADC, is comprised of an indolino-benzodiazepine mono-imine DNA-alkylating payload, DGN462, coupled at lysine residues by a cleavable N-succinimidyl 4-(2-pyridyldithio)-2-sulfobutanoate (s-SPDB) linker to a novel huCD33-targeting antibody (Z4681A), with an average drug: antibody ratio (DAR) of 2.7.

IMGN779 is currently undergoing clinical evaluation in adult patients with relapsed/refractory CD33-positive acute myeloid leukemia.

Methods

An anti-human CD33 monoclonal murine antibody (My9-6) was selected and humanized by amino acid resurfacing, resulting in the Z4681A antibody used in all the CD33-targeting ADCs.

Free mono- and di-imine payloads were evaluated *in vitro* for cytotoxicity on human AML cell lines by culturing cells for 72 hr either with serial dilutions of payload or with formulation buffer, followed by the addition of WST-8. After 2-8 hr of WST-8 exposure, absorbance at 450 nm was determined.

Payloads were compared *in vitro* for cytotoxicity on human AML cell lines and *in vivo* for single IV dose tolerability in CD-1 mice and for efficacy against human AML xenografts (HL60, SC implantation, single IV injection) as part of huCD33-targeting conjugates. For mouse tolerability, BW was determined, and 20% BW loss or day 90 were the study endpoints. For efficacy, SC tumor volumes were measured twice a week; and clinical signs and BW loss were the endpoints for disseminated models.

ADCs utilizing the DGN462 DNA mono-alkylating payload, conjugated to the huCD33 antibody with cleavable or non-cleavable linkers, were evaluated for cytotoxicity on MDR-positive AML cell lines (HEL92.1.7, OCI-M1), single-dose tolerability in CD-1 mice and efficacy in AML xenografts (HL60, SC, single IV injection).

IMGN779 was also evaluated *in vivo* for single-dose toxicity in rats and cynomolgus monkeys. The antitumor activity of IMGN779 was evaluated in AML xenograft models in multi-dose regimens in disseminated models (MV4-11, Molm-13) and in fractionated dose regimens (MV4-11, SC).

Abbreviations: BW, body weight; IV, intravenous; MTD, maximum tolerated dose; MED, minimum effective dose; SC, subcutaneous

DGN462 (mono-imine, DNA mono-alkylator) and DGN484 (di-imine, DNA cross-linker) payloads have comparable IC50s as free drugs and as CD33-targeting conjugates

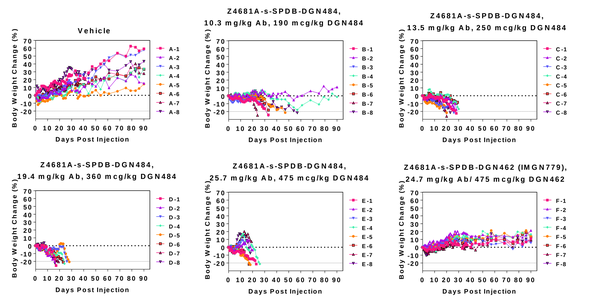
AML Cell Line	DGN462 Alkylator Free Drug IC50 (pM)	DGN484 Cross-Linker Free Drug IC50 (pM)
EOL-1	12	5
HL60	260	59
OCI-M1	140	77

AML Cell Line	Z4681A-DGN462 Alkylator ADC IC50 (pM)	Z4681A-DGN484 Cross-linker ADC IC50 (pM)
EOL-1	6.5	2.2
HNT-34	36.1	6.7
MOLM-13	0.73	0.46
MV4-11	3.5	1.1

- **ADCs utilized the cleavable -s-SPDB- linker.**
- **ADC concentrations are by antibody.**

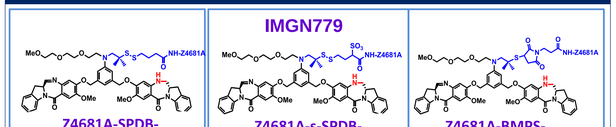
DNA mono-alkylating payload (DGN462) is better tolerated than DNA cross-linking payload (DGN484)

ADC	MTD in Mice (DGN; Ab)	MED in AML Xenograft (DGN; Ab)	Therapeutic Index (TI)
Z4681A-s-SPDB-DGN462 (IMGN779)	950 µg/kg; 48.1 mg/kg	10 µg/kg; 0.522 mg/kg	95-92
Z4681A-s-SPDB-DGN484	190 µg/kg; 10.3 mg/kg	10 µg/kg; 0.541 mg/kg	19



- **Mice dosed with Z4681A-DGN484 (DNA cross-linker ADC) at > 190 µg/kg (by DGN484) reach BW endpoint on days 17 to 28**
- **Delayed toxicity (endpoints on days 33-57) evident in mice dosed with 190 µg/kg Z4681A-DGN484**
- **No significant BW loss in mice dosed with 475 µg/kg IMGN779 (DGN462)**

Cleavable linker formats were better tolerated than non-cleavable linker



Z4681A-SPDB-DGN462

- Cleavable Linker
- MTD: 700 µg/kg
- MED: 20 µg/kg
- TI: 35
- *In vitro* activity (MDR+)
  - HEL92.1.7: 3 nM
  - OCI-M1: 3 nM

IMGN779

Z4681A-s-SPDB-DGN462

- Cleavable Linker
- MTD: 950 µg/kg
- MED: 10 µg/kg
- TI: 95
- *In vitro* activity (MDR+)
  - HEL92.1.7: 0.2 nM
  - OCI-M1: 0.5 nM

Z4681A-BMPS-DGN462

- Non-cleavable Linker
- MTD: 284 µg/kg
- MED: 20 µg/kg
- TI: 14
- *In vitro* activity (MDR+)
  - HEL92.1.7: 0.03 nM
  - OCI-M1: 0.03 nM

- **MTD in CD-1 mice, single dose (µg/kg DGN462)**
- **MED in HL60 AML xenograft model, single dose (µg/kg DGN462)**
- **TI = MTD/ MED**
- ***In vitro* IC50**

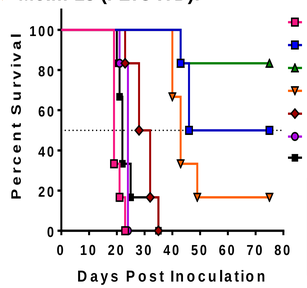
IMGN779 (Z4681A-s-SPDB-DGN462) at MTD has a consistent toxicity profile across multiple species

Parameters Examined		Species-Related Findings: Mouse	Rat	Monkey
Liver Enzymes	ALT, AST, SDH	Mild elevation	Slight decrease ALT/ALP, slight elevation SDH	No changes.
Decreases in Blood Cell Counts	Thrombocytes	Mild to Moderate	Mild to Moderate	Mild to Moderate
	Reticulocytes	Severe	Severe	Severe
	Neutrophils	Moderate	Moderate to Severe	Moderate to Severe
	Lymphocytes	Moderate	Moderate	Mild
Clinical Observations		Body weight loss	Body weight loss (diarrhea at doses > MTD)	Body weight loss/ GI toxicity/ diarrhea
Histopathology		Gross/ microscopic: GI damage/ bone marrow depletion	Gross/ microscopic: GI damage/ bone marrow depletion	Gross/ microscopic: GI damage/ bone marrow depletion

- Single dose

IMGN779 is highly active against disseminated AML xenografts

Molm-13 (FLT3-ITD):

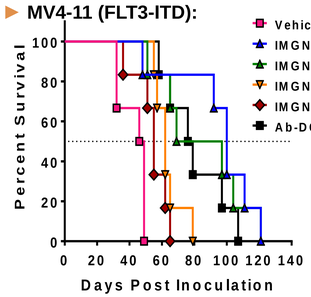


QW x 3

MED = 0.00534 mg/kg Ab; 0.1 µg/kg DGN462

Treatment Group µg/kg DGN462/ Dose, QW x 3	Median Survival Time (Days)	% Increased Life Span	Result
Vehicle, 0	19	---	---
IMGN779, 10	> 75	> 295	Highly Active
IMGN779, 3	> 75	> 295	Highly Active
IMGN779, 1	43	126	Highly Active
IMGN779, 0.3	30	58	Highly Active
IMGN779, 0.1	24	26	Minimally Active
Ab-DGN462, 10	22	16	Inactive

MV4-11 (FLT3-ITD):



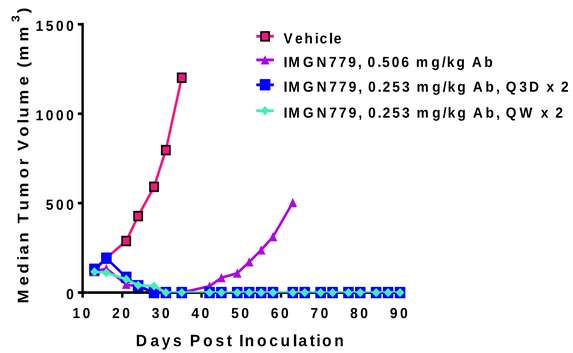
QW x 3

MED = 0.0534 mg/kg Ab; 1 µg/kg DGN462

Treatment Group µg/kg DGN462/ Dose, QW x 3	Median Survival Time (Days)	% Increased Life Span	Result
Vehicle, 0	48	---	---
IMGN779, 10	100	108	Highly Active
IMGN779, 3	83	73	Highly Active
IMGN779, 1	64	33	Minimally Active
IMGN779, 0.3	55	15	Inactive
Ab-DGN462, 10	77.5	61.5	Highly Active

Additional benefit derived from fractionated IMGN779 dosing against AML xenografts

MV4-11 (FLT3-ITD):



Treatment Group	% T/C (Day 31)	Tumor Free Survivors (Day 90)	Result
Vehicle	---	0/6	---
IMGN779 (10 µg/kg DGN462)	0	3/6	Highly Active
IMGN779 (5 µg/kg DGN462; days 1,4)	0	5/6	Highly Active
IMGN779 (5 µg/kg DGN462; days 1,8)	0	5/6	Highly Active

SUMMARY

- DNA mono-alkylating payload confers higher MTD, with similar MED, compared to DNA cross-linking payload, resulting in higher TI in xenograft models.
- Cleavable linker format confers higher MTD than non-cleavable linker format.
- IMGN779, designed as a next generation CD33-targeting ADC, utilizes a novel DNA mono-alkylating DGN462 payload and a cleavable disulfide linker, combining components selected to maximize anti-AML activity and preclinical safety.
- IMGN779 is highly active and well-tolerated in preclinical repeat-dosing regimens.
- IMGN779 is highly active in multiple AML xenograft models, including models with poor prognostic factors.
- IMGN779 dose fractionation provides an additional long-term benefit over treating AML xenografts with a single high dose.
- These results provide the foundation for the clinical evaluation of IMGN779 in AML.

Clinical testing is ongoing: NCT02674763: Open-label Study of IMGN779 in Adult Patients With Relapsed/Refractory CD33-positive Acute Myeloid Leukemia. Clinical Poster: P562, Saturday, June 24; 17:30 to 19:00, Hall 7

EHA, June 22-25, Madrid, Spain